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Doppler ultrasound and other imaging modalities have been used to assess characteristics of vasculature associated with malignant breast masses. 3D contrast refill imaging should help visualize slow-flow in small neo-vasculature associated with these masses. The dual-transducer method proposed should provide vascular mapping while minimizing acquisition time, the major limitation of techniques such as interval-imaging (I-I) and real-time (RT) imaging.

Phantom tube-flow studies from Y2 were further quantified. A phantom kidney model initially tested in Y2 has been extensively studied and compared to I-I and RT methods. Image volumes reconstructed using the dual-transducer method displayed remarkable spatial detail. After accounting for contrast decay, mean transit times (MTTs) for image planes derived from reconstructed image volumes were consistent ($p<0.05$) with corresponding single I-I and RT scans. In addition, the development of a parametric image display scheme has been undertaken. Raw images highlighting differences in MTT throughout the model have been generated, and refinements to parametric calculations are currently underway.

Limitations of the clinical ultrasound machine were still problematic during Y3, and no patient data has been acquired. We hope to conduct a slightly abridged clinical phase during a no-cost extension period. Y3 results are presented here.

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Introduction

The overall objective of this project has been to develop a 3D ultrasound contrast imaging system for characterizing suspicious breast masses. The originally proposed method uses a dual-transducer scheme to map mean blood transit time in three dimensions. The method requires a fraction of the time necessary to obtain similar information using other standard contrast imaging techniques, and it should provide information related to normal and anomalous vascular characteristics in and around suspicious masses. In addition, the technique should allow visualization of areas of slow flow and microvasculature, which cannot be detected with conventional Doppler imaging methods. It is hypothesized that these measures will enhance our ability to discriminate benign from malignant lesions as well as serve to increase our understanding of tumor biology in terms of vessel formation.

The originally proposed schedule would have concluded at the end of year three; however, a no-cost extension was added for further refinements and clinical trials. As such, year three was used primarily for laboratory experimentation and data analysis to understand the 3D contrast imaging. Although the dual-transducer method facilitates full 3D contrast imaging when other slice-by-slice interval-imaging or so-called real-time imaging schemes would be time prohibitive, the method still does require some finite time, enough to influence results during experiments with slow-flow models. The experimental artifacts encountered were presumably due to contrast decay. These effects are discussed in the report. Additionally, dual-transducer method results from earlier tube-flow experiments are further quantified, and 3D imaging results from a kidney phantom are compared with other imaging schemes. Ramifications of contrast decay *in vivo* are also briefly discussed.

We are now able to cardiac-gate the GE Logic 9, and basic manipulation of a transducer scanning apparatus is possible; however, there remain a few technical issues in fully implementing a modified dual-sweep method for contrast imaging in the clinical setting. From the software imaging standpoint, an initial parametric flow imaging scheme was developed and applied to 3D kidney images, and an example is shown. Refinement of this imaging scheme is currently being refined. Given the continued delay in patient scanning, we are hoping for a small no-cost extension to the project, which should allow for some concrete clinical results.

Body

Background:

Given that the following background text provides information regarding the impetus of the proposed work, it is essentially unchanged from our previous reports. As mentioned in the original proposal and other yearly reports, previous studies by other investigators have

demonstrated characteristics of vasculature associated with malignant breast masses. These have included thin-walled blood vessels, increased microvessel density, disordered neo-vascularization penetrating the mass, arteriovenous shunting, and a variety of characteristic Doppler ultrasound and histologic findings [Lee et al. 1996, Peters-Engl et al. 1998]. Some studies strongly suggest that flow velocity demonstrates significant correlation with tumor size [Peters-Engl et al. 1998] and that parameters such as vessel count and flow velocity display significant differences between malignant and benign lesions [Madjar et al. 1994]. A shortcoming of most of these trials has been the limitation of 2D images in assessing overall vascular morphology, density, and velocity distributions.

Given the limitation of 2D studies and the relative sparseness of breast vasculature, our group has investigated the utility of 3D breast imaging for several years. Recently published results [LeCarpentier et al. 1999] indicate that one of our Doppler vascularity measures, Speed Weighted pixel Density (SWD), is statistically different for benign versus malignant lesions and comparable to ultrasound grayscale (GS) evaluation. More recent work in a 38 patient pool suggests that multi-variable indices (which include both SWD and GS features) demonstrate good results in differentiating benign from malignant breast masses well beyond GS evaluation alone [Bhatti et al. 2000]. In a follow-up study (submitted and accepted for publication), the results of the initial 38 patients (18 benign, 20 malignant) were used to form a learning set (A), and multivariable indices were established using Bayesian discriminators. In Group A, 94% specificity was achieved for the SWD-Age-GS index at 100% sensitivity. Applying the same linear function to the second pool (B) resulted in 86% specificity at sensitivity of 100% [LeCarpentier et al. 2002]. The diagnostic performance of SWD in our second patient population strongly suggests the utility of vascular indices in the characterization of breast masses.

In addition to Doppler imaging, a number of investigators have performed extensive evaluation of ultrasound contrast agents in the evaluation of blood flow. Success of low-frame-rate imaging (termed "transient response imaging" or "interval imaging") is related to the "refill" of agent into tissue [Porter and Xie 1995, Porter et al. 1997]. Monitoring refilling has estimated the perfusion in tissue [Wei et al. 1998] and specific pulsing sequences such as "Flash Echo Imaging" (Toshiba Medical Systems) and "Power Pulse Inversion" (ATL/Phillips) have been developed on ultrasound scanners to obtain refill information. Studies at our institution [Fowlkes et al. 1998] have shown that it is possible to destroy contrast agent flow in arteries to produce interruptions with signal separation up to 30 dB. Similar interruptions allow downstream contrast agent to clear and the release of a short bolus by temporarily turning off the field [Rhee et al. 1998]. All of these methods rely on controlled destruction of contrast agents and subsequent reflow into tissue. Complications associated with such measurements in 3D are addressed in this work.

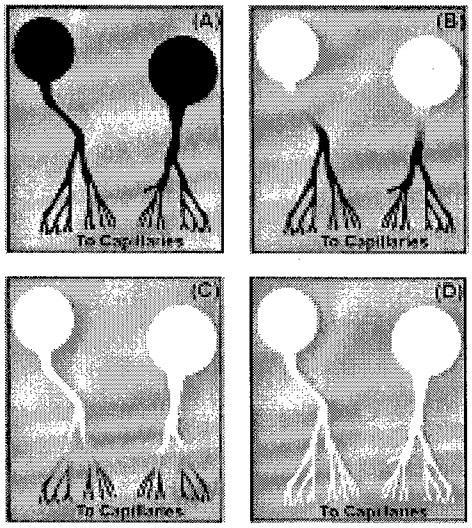

Figure1. Schematic representation of ultrasound contrast refill. An ultrasound beam is used to destroy contrast agent in all vessels in the imaging plane (A). The larger vessels quickly refill (B) and feed the smaller arteries and arterioles with fresh contrast (C & D). Over time, capillary refill can be visualized.

Figure 1 shows a general schematic of contrast disruption and refill. An ultrasound beam is used to destroy contrast agent in all vessels in the imaging plane. The larger vessels with significant volume flow and high flow rates would quickly refill. The volumes of interest, however, are slower flow in the capillary bed. As the arterioles are filled, the contrast can be visualized, and eventually capillary refill will be seen. Figure 2 depicts the dual-transducer imaging scheme. For the sake of discussion, consider the case of a patient under constant drip infusion of ultrasound contrast agent. At steady state, the imaged blood is 100% contrast enhanced. By translating an ultrasound transducer transmitting a sequence of high-intensity pulses, a wavefront of maximally broken contrast or "zero-contrast-enhanced" tissue is formed. Although the figure shows vessels virtually flowing in the same direction for simplicity, a model will be developed to describe the more "real-world" scenario of isotropically distributed flow into and out of the particular volume of interest. The second transducer, which arrives at the same location at time 2, images the partially refilled volume. This process can be repeated multiple times using different delay settings between contrast destruction and imaging to estimate refill rates for every region in the overall imaged volume.

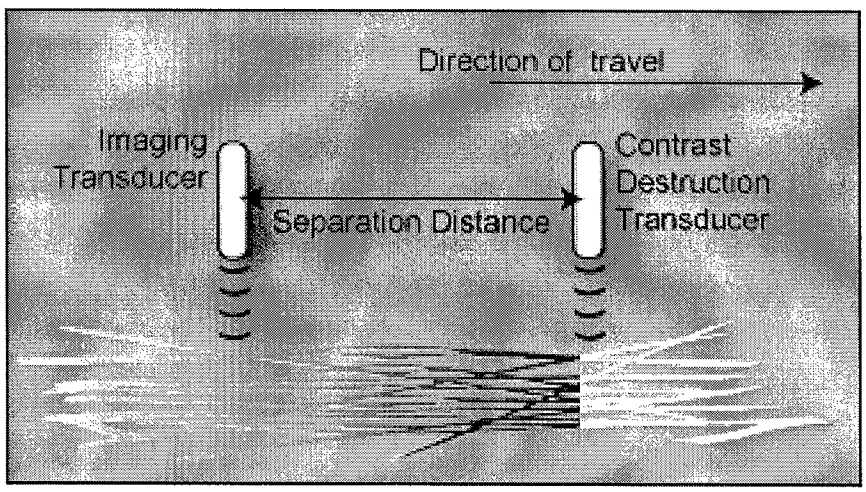

Figure 2. Schematic of dual-transducer method or monitoring capillary refill. The high intensity transducer is used to destroy contrast and create a zero-contrast-enhanced wavefront. The low intensity imaging transducer follows behind at some fixed time (distance). By sequentially scanning the same region using different delays, a refill-time map can be constructed for the volume.

Specific Tasks:

In the originally proposal document, the approved statement of work included the five major tasks listed below:

Task 1 (months 1-6): Model input function of contrast agent destruction:

(a) Generate mathematical flow model

(b) Measure beam profile

(c) Incorporate various profiles, flow, and scan rates

Task 2 (months 3-12): Assemble and test mechanical imaging scan system:

(a) Design and construct mechanical translation system

(b) Design and test electrical interface

(c) Design and test interface software

Task 3 (months 13-24): Design and perform experimental assessment of imaging system design:

(a) Evaluate performance on strict flow tube models

(b) Evaluate performance on kidney phantom

(c) Evaluate 3 point method of refill curve modelling

Task 4 (months 1-24): Develop and assess visualization and quantification software:

(a) Verify flow model

(b) Develop regional mapping software (*can start as soon as the project begins)

(c) Develop and evaluate parametric histogram visualization scheme

Task 5 (months 13-36): Assess system and 3D imaging software on small patient population:

(a) Recruit patients

(b) Perform scans

(c) Evaluate refill maps and parameterize

(d) Test discriminators

Task 6 (months 30-36): Overall data analysis and write-up

Given that the third year was operating under the assumption of a no-cost time extension, it is difficult to estimate exactly how the flow of the project relates to the original outline. Nonetheless, significant progress has been made working out the logistics of the method itself, characterizing contrast flow in phantom models, and in establishing initial parametric image schemes (Tasks 3 & 4 above). The results of tube flow experiments were further characterized, and extensive experimental methods were applied to porcine kidney phantoms. In addition to what was initially proposed, characteristics of the contrast agent itself were analyzed, and a scheme was developed to correct for artifacts due to long-term contrast instability.

As for assessing the device on a patient population, we opted to stay in the laboratory until the aforementioned characteristics were appropriately analyzed. There were additionally a few technical issues which remained to be addressed regarding a dual-sweep method implementation in the clinic. We have been (appropriately) hesitant to enter clinical trials before better understanding contrast degradation as well as developing a fast time-effective

protocol, which is currently underway. We still do hope to obtain a small second no-cost extension to assess the method on a small patient pool.

Results:

A laboratory set-up and interface software was developed to implement the dual-transducer method described in the introduction and previous report. The apparatus shown in Figure 3 was used to translate the transducer pair at a constant rate. The distance between the two transducers was varied, and clearance/imaging sequences were performed over a 6.35 mm flow tube and kidney phantom.

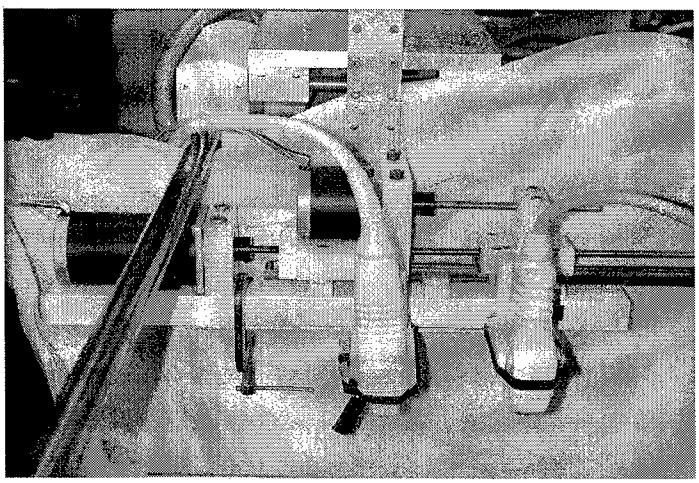

Figure 3. Dual-transducer apparatus. Two stepper motors turn two threaded rods parallel to each other to move the ultrasound transducers, held by the transducer holders which translate on a guided slide. The separation motor, mounted upon a stand, adjusts transducer s eparation. The stand in turn is translated by the larger translation motor, which controls the sweeps over the volume of interest. For the experiments presented, the complete apparatus was placed within a rubber-lined water tank upon a stand, and cables connect the motors to a control box (not shown), which provided drive power and was in turn controlled by a laptop computer using the DAQCard-700 and LabVIEW scripts.

As described in last year's report, parabolic flow profiles were expected in slow tube flow scenarios. Briefly: Laminar, viscous fluid flow velocity (V) through a straight, circular tube has a parabolic velocity profile as a function of radial distance (r) from the center given by

$$V(r) = Vmax \bullet (1 - (r/R)^2)$$

where $Vmax$ is the peak velocity and R is the tube radius. If one were to take a "snapshot" of the flow pattern at any given moment, this pure parabolic profile would be seen. In the case of the dual-transducer method, however, each slice is actually acquired at a different time, hence creating a "time-dilated" parabola upon imaging. If one solves for the position (distance from the starting point, d) of the parabola "front" as a function of time, the expression is simply

$$d(t,r) = t \bullet V(r).$$

Substituting the previous equation and solving for r yields

$$r = R \bullet \mathrm{sqrt}(1 - d/t \bullet Vmax).$$

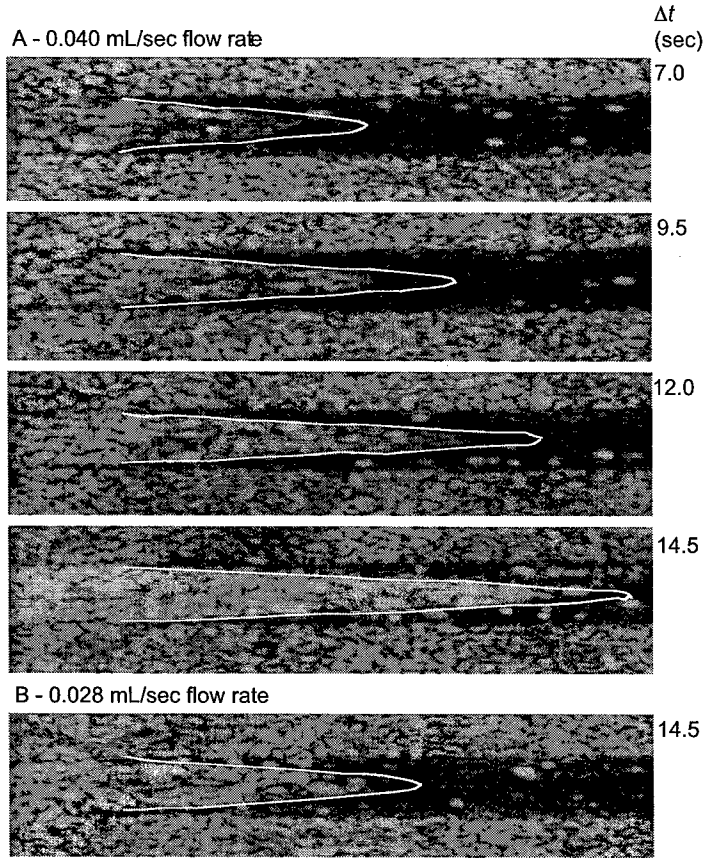

Figure 4. Reconstructed images along the length of the flow tube showing the contrast agent flow. The white line shows the theoretical position of the contrast front. The flow passed through a tube of radius 3.175 mm at a rate of 0.040 mL/sec (A) and 0.028 mL/sec (B). Transducer translation velocity vT was 6 mm/sec. Expected profiles closely match those observed, except at the profile tip. It is believed that the tip discrepancy is due to slight contrast destruction due to both increased beam exposure at the tube center and decreased overlying attenuation from overlying contrast.

Time-dilated parabolas of this form were calculated and super-imposed over experimental results and are presented in Figure 4. We have since quantified the measured flow by using hand traces of the contrast edge to generate new measured quasi-paraboloids such that Vc estimates could be calculated from these fits. The resultant Vc estimates are presented in Figure 5. The Vc under-estimate in most cases is due to the truncation of the leading edge of the contrast front, likely due to contrast clearance along the central axis, as well as the absence of overlying attenuation at the far extent of the curve (explained further in Y2 report). Nonetheless, Vc estimates were still within 15% of their expected values.

We continued studies on fixed porcine kidney phantoms as described by Holmes and others [Holmes et al. 1984]. As mentioned previously, the logistics of dehydrating and rehydrating these kidneys were initially problematic. After overcoming these difficulties, a flow system was assembled and tested. The stability of contrast agent (Definity) was also problematic given the relatively long (20-30 minute) experimental protocol due to contrast exposure to atmosphere, suspension (stirring) issues, and various pumping parameters. Experimental results were thus interpreted with all of these factors in mind.

Figure 6 shows representative transverse images of contrast imaging during various imaging scenarios. From top to bottom, interval imaging (II) is shown for 100% and 2% acoustic output, followed by a "real-time" imaging sequence, and finally the dual transducer case. Note

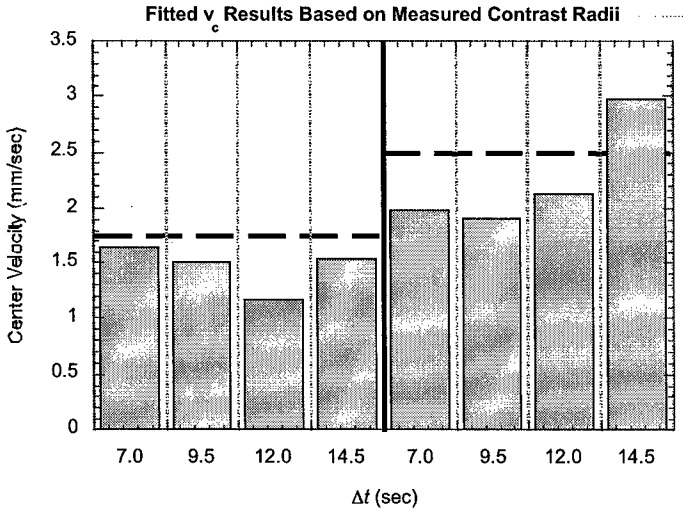

Figure 5. Fitted Vc results for cylindrical tube phantom. Dual transducer scans were acquired for the cylindrical tube phantom with two center flow velocities (1.75 and 2.50 mm/sec - corresponding to the flow rates of 0.0028 and 0.0040 mL/sec respectively from Figure 4) using four different refill times. Shown are the measured values of Vc obtained by fitting measured contrast radii to the equation of the flow parabaloid. Dashed lines denote theoretically known values of Vc. Measured values of Vc are on average 13.2% less than those expected.

the similarity in the image sequences. The actual refill curves generated by these image sequences are displayed in Figure 7. The figure suggests a problem with contrast agent decay.

The contrast decay alluded to in Figure 7 was used to adjust subsequent analyses. In the case of "real-time" imaging, the acoustic output (AO) of the imaging transducer was 2%. Similarly, AO in one of the II scenarios was the same. Additionally, interval imaging relies on essentially the same geometry and clearance/refill characteristics as real-time imaging, so one might expect similar results for the two schemes, the only difference being the time required to acquire the II data. As such, we determined the contrast decay based on equalizing contrast mean transit times (MTTs) during RT and 2% II modes. Results are shown in Figure 8, showing that 4.1%/min contrast decay rate equalizes RT and II curves taken over relatively short (~4 minutes) and long (~25 minute) time frames. Using intensity levels obtained for this equalization, contrast decay rates were determined for other test scenarios. In these cases, contrast decay rate was adjusted such that the mean MTT determined for RT and II scenarios fell within the 95% confidence interval of the particular scenario (various dual transducer runs and II at 100%). Mean "adjustments" were on the order of a 5%/minute decay rate. This was deemed well within previously observed contrast decay values. Thus, the DT technique proved consistent with other standard 2D imaging methods.

Figure 9 shows images similar to those of Figure 6. In this case, however, II and RT images were acquired at a transducer angle 90° from those acquired previously. DT images were extracted from a full 3D image volume by registering the longitudinal RT image plane with the volume via UofM developed software MIAMIFuse. Figure 10 demonstrates results similar to those shown in Figure 8, this time comparing a DT image plane extracted from the reconstructed volume to the directly measured RT and II images.

Figure 10 demonstrates our initial effort in displaying refill parameters in the image.

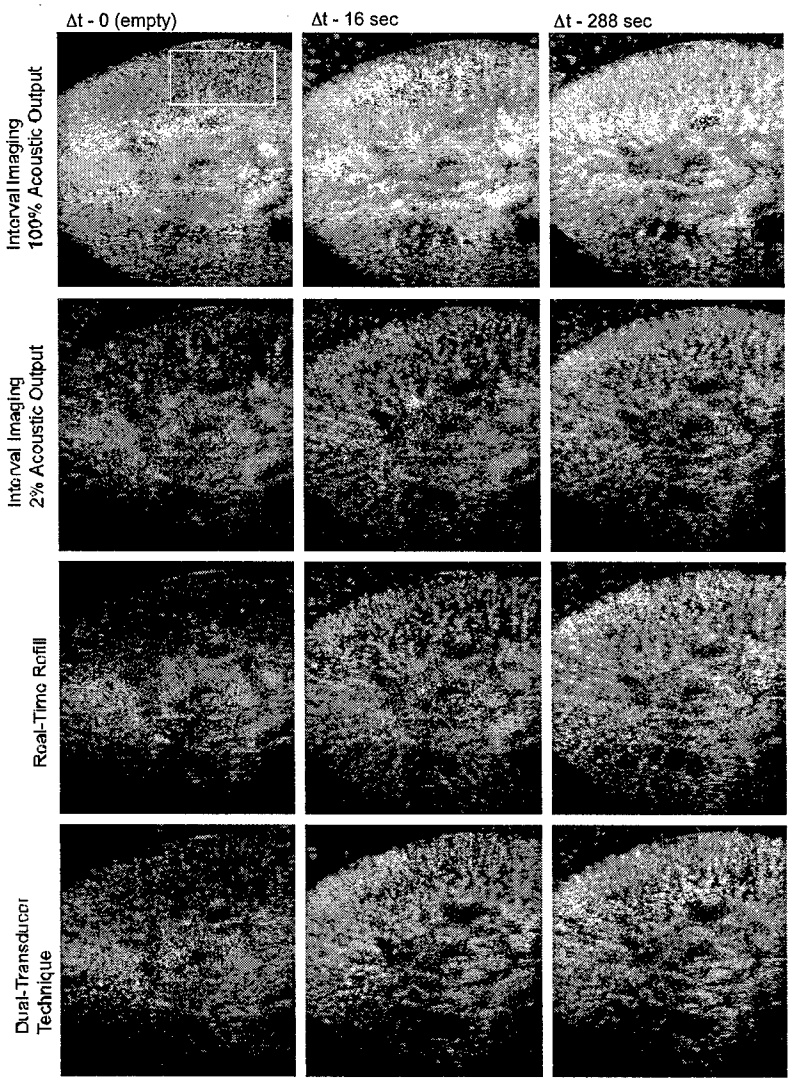

Figure 6. Representative transverse images obtained for a representative slice from preserved porcine kidney with interval imaging, real-time refill, and the dual transducer technique for refill times of 0, 16, and 288 sec. Images from real-time refill and dual transducer are obtained with the same acoustic settings as the 2% acoustic output interval imaging data. Interval imaging images obtained at 100% acoustic output are brighter because of the high transducer output used to obtain the images. Contrast clearance was obtained for both interval imaging cases and real-time refill using 50 pulses at 5 Hz at an acoustic output of 100%. The separate clearance transducer provided contrast clearance for the dual-transducer measurements. A rectangular window marks the region of interest in subsequent mean transit time comparisons.

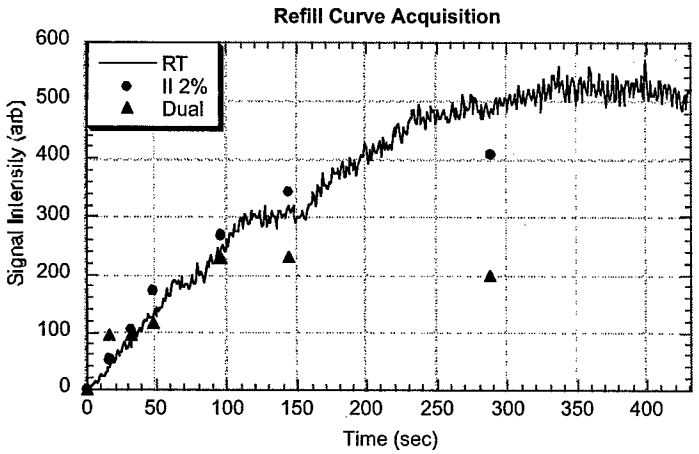

Figure 7. Example of measured refill curves. Abbreviations used are as follows: II - Interval Imaging, RT - Real-Time refill, DT - Dual Transducer technique, MTT - Mean Transit Time. Real-time refill imaging provides nearly continuous data for the ROI. Interval imaging and the dual transducer technique provide specific time-point measurements to curve fit and estimate MTT. The II acquisition was obtained at an acoustic output of 2%. Fitted MTT results (in sec) for these curves are RT - 182.23, II - 101.44, and DT - 44.55. The earlier saturation and subsequent signal drop of the II and DT cases are probably due to contrast degradation over time.

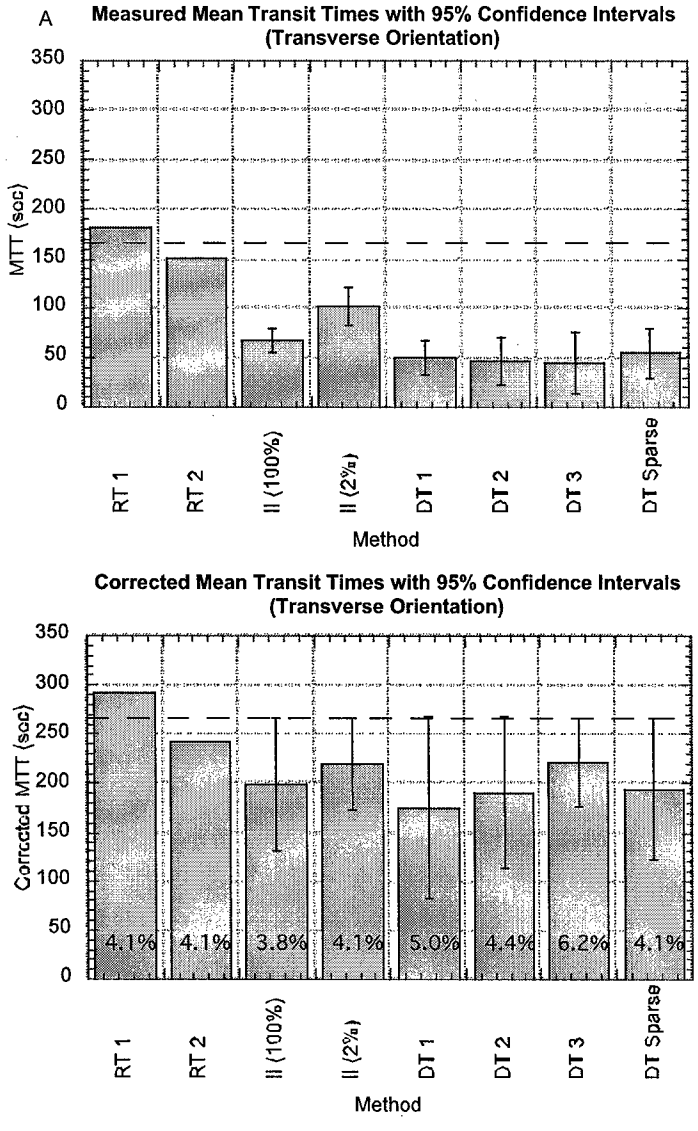

Figure 8. (A) Comparison of data obtained for a given transverse slice, with the ROI (regions of interest) indicated in Figure 6. Numerals refer to repeated acquisitions using the stated method (e.g. DT 3 - third acquired dual transducer data set). Error bars indicate the 95% confidence interval of the mean transit time value, as fitted to the expression A[1-exp(-1/MTT)] for measured data points. Percentages (for II) refer to the acoustic output (AO) used for readout after set of clearance pulses. Sparse refers to the acquisition where images were obtained at a large (20 mm) slice separation. (B) Factoring in the degradation of contrast agent signal with time, the MTTs can be corrected to values within statistical significance (see text). Because the RT and II measurements are at 2% AO with identical clearance pulse schemes, their MTT measurements are expected to be very similar. Correcting both of these with a contrast degradation factor of 4.1%/min would make the II MTT value statistically equal to the mean of the RT measurements. Applying correction factors (in %/min) for the other measurements as indicated would cause the RT MTT mean to be within their 95% confidence interval.

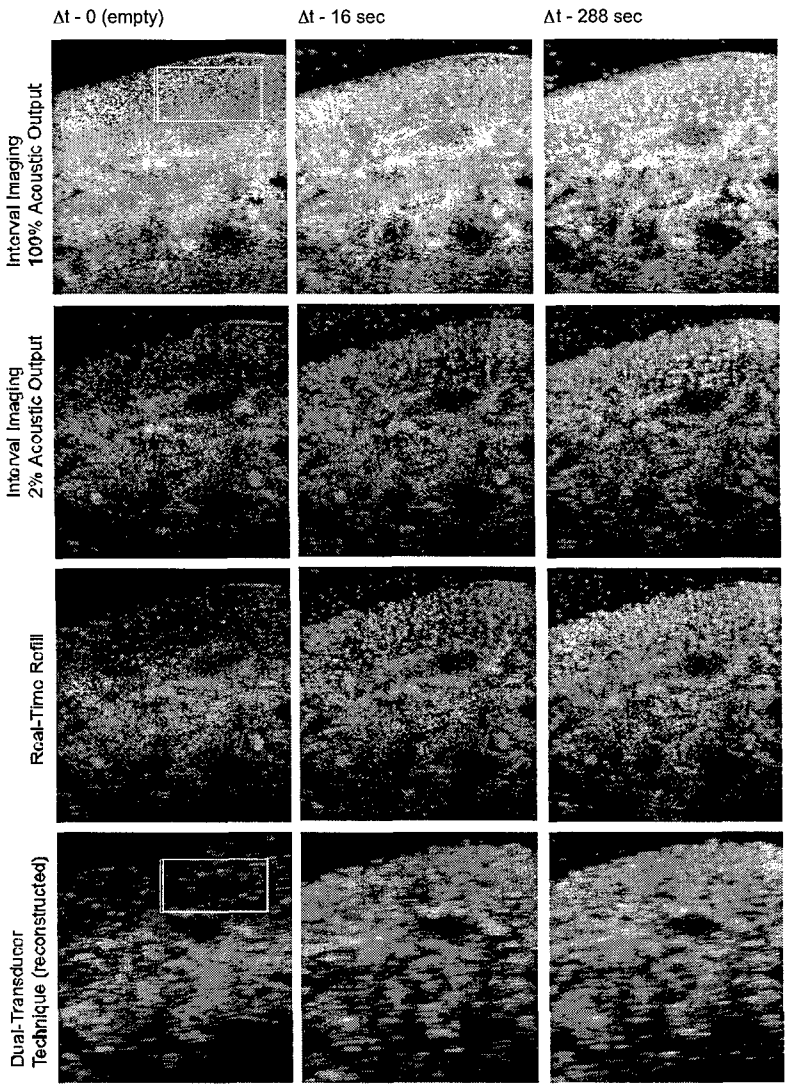

Figure 9. Representative longitudinal kidney images analogous to those in Figure 6. The kidney was rotated 90° and had interval refill data (at 100% and 2% acoustic output) and real-time refill measurements taken for the slice plane shown. Refill images for 0, 16, and 288 sec are displayed, with the ROI marked by a rectangular window. Contrast clearance was achieved with 50 100% AO pulses at 5 Hz. The dual transducer images were obtained by stacking the transverse images from the dual-transducer scans in the elevational direction, and selecting the plane closest to that seen with the perpendicular scans through image registration with in-house (MIAMI Fuse) registration software. The images obtained are of a lower quality because of both the slice separation of 1 mm, and the transducer point-spread function in the elevational direction. However, one still observes contrast filling with time. The marked ROI is that which corresponds closest to the ROI marked in the directly acquired perpendicular images.

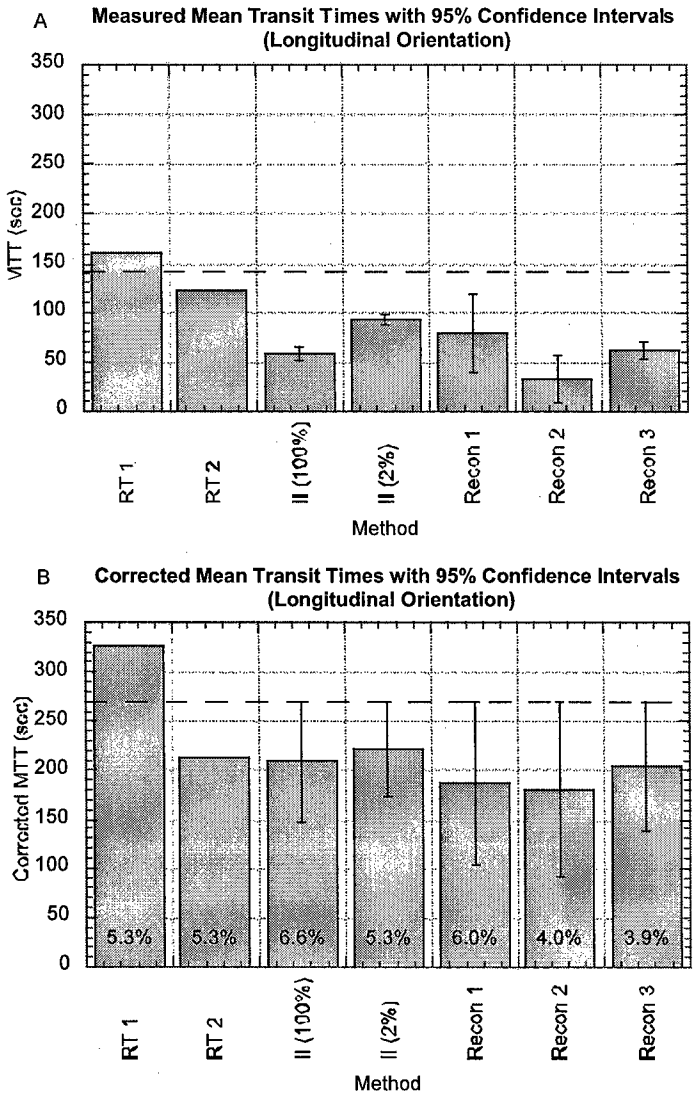

Figure 10. (A) Comparison of mean transit time (MTT) data for perpendicular kidney images with the ROI as indicated in Figure 9. Recon refers to the images formed from the transverse dual transducer scans. MTT trends are similar to those in Figure 8 for analogous reasons. Dual transducer data was consistent among the three acquisitions. Error bars refer to the 95% confidence interval of the curve fit MTT. (B) Results obtained after applying a correction factor analogous to that in Figure 8B. Applying a correction for degradation of 5.3%/min makes the II curve acquired at 2% AO statistically equal to the mean of the corresponding RT measurements. The other measurements require correction factors as indicated to yield MTTs equal to the mean of the RT measurements.

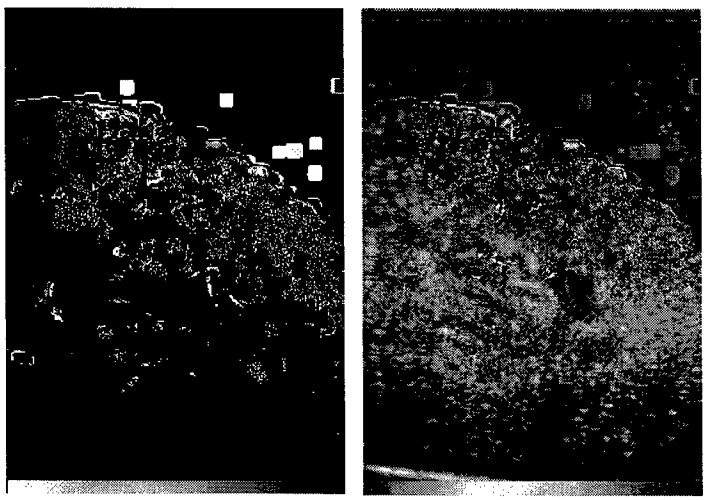

Figure 11. Parametric images of mean transit time (MTT) in porcine kidney. (Left) Grayscale bar white to nearly black represents shortest MTT (fastest refill) to longest MTT (slowest refill). Indeterminant points are black. (Right) Colorized MTT super-imposed onto original image, red to blue represents fastest to slowest refill. Efforts are underway to reduce the indeterminant regions, apparent primarily in the medullary region in this example.

Key Research Accomplishments

- Tube flow characteristics from previous year were further quantified using curve fits to hand selected contrast edge profiles. Despite truncation of the profile due to excessive beam exposure at the center and distal end of the tube, flow estimates were still within 15% of their presumed value.

- Experimental apparatus was extensively tested on tube flow models and porcine kidney phantoms. Contrast decay was noted under a variety of experimental conditions, and attempts were made to minimize these artifacts.

- Flow measurements throughout the entire volume in kidney phantoms were obtained using the dual-transducer (DT), and mean transit times (MTTs) were calculated. Selected image planes were compared with MTTs acquired in similar planes using real-time (RT) and interval-imaging (II) methods.

- In-house UofM software (MiamiFuse) was used to extract from the 3D DT volumes longitudinal image planes (i.e. perpendicular to original image planes), which were compared to longitudinally acquired II and RT images.

- A correction scheme was developed to account for remaining contrast decay artifacts, and estimate decay rates during the DT method. Resultant calculated decay rates appear at least consistent with previous observations in vitro, on the order of 4% per minute. Assuming decays of this magnitude, DT results were consistent with II and RT imaging methods.

- Initial parametric flow images were generated. Work is ongoing to avoid "indeterminate" regions, particularly areas with slower flow.

- Hardware development for a modified dual-sweep approach to *in vivo* clinical studies is underway.

Reportable Outcomes

Their were no new papers or presentations during the third year of the proposed research. However, the following is in preparation:

A Dual Transducer Technique for Visualizing Three-Dimensional Refill Data

As of this writing, the following abstracts encompassing two different aspects of the project were submitted for review:

Chen NG, Fowlkes JB, Carson PL, LeCarpentier GL. Assessment of a 3D Dual-Transducer Ultrasound Contrast Agent Technique for Vascular Imaging. BMES Annual Meeting, Baltimore, 2005 (submitted).

Chen NG, Fowlkes JB, Carson PL, LeCarpentier GL. A 3D Dual-Transducer Ultrasound Technique for the Assessment of Vascular Flow Using Contrast Agent Imaging. RSNA, 91st Scientific Assembly and Annual Meeting, Chicago, 2005 (submitted).

Conclusions

The dual-transducer technique provides vascular refill information highly correlated to interval and real time imaging, while drastically reducing imaging time required for a 3D volume. The technique may provide measures of tissue perfusion and refill characteristics which are unobtainable with current Doppler methods, although our previous Doppler analysis methods are well suited to contrast agent imaging quantification and breast mass characterization.

There is a time "cost" in obtaining refill data via any clearance/imaging scheme (whether it be interval imaging in 2D or the dual transducer method in 3D), and slow-flow regions may be affected by contrast decay. *In vitro,* such effects might be accounted for by monitoring contrast signal levels in a major vessel between sweeps and normalizing subsequent sweeps accordingly. In practice, however, refill times of less than a minute (which is more typical for the *in vivo* case than our delicate slow flow kidney model) may be relatively unaffected by contrast decay.

We have been encouraged by the success in obtaining full 3D refill image volumes, and expect that a transition into a small clinical trial is forthcoming. We hope to then enhance our ability to detect slow flow and possible neo-vascularization, which could in the long run aid in our understanding of changes in microvasculature at early stages of tumor development.

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